HYPOGLYCEMIC ACTIVITY OF ETHANOLIC EXTRACT AND FRACTION OF MENGKUDU (Morinda citrifolia L) FRUITS IN GLUCOSE INDUCED RATS

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ABSTRACT

Objective: To evaluate the hypoglycemic activity of ethanolic extract and fraction of Mengkudu (Morinda citrifolia L.) fruits in rats (Wistar) induced glucose and that the fruit used empirically to treat hyperglycemia in Indonesia, in order to determine ethanolic extract and fraction with the most potential as a hypoglycemic agent.

Methods: Hypoglycemic activity test was conducted in healthly Wistar rats, glucose induced male rats with glucose tolerance test. The ethanolic extracts with three variation doses at 0,3; 06 and 1,2 g /kg bw and at the same doses 1,2/kg bw of activity of water, ethyl acetat (base), ethyl acetate (acid), and n-hexane fraction of Mengkudu (Morinda citrifolia L) fruits given orally. The blood samples were used for determination of glucose level examination using spectrofotometric with GOD-PAP methode.

Results: The ethanolic extract of Mengkudu (Morinda citrifolia L.) fruits has the best activity in reduction of blood glucose level in rats at dose 1,2 g/kg bw, followed by dose 0,6 g/kg bw and dose 0,3 g/kg bw and also showed that the aethyl acetate (acid) fraction gave the best activity in reduction of blood glucose level in rats (54,29%), followed by water fraction (47,42%), ethyl acetate (base) fraction (44,51%) and n-hexane fraction (34,18%).

Conclusion: Results of the present study suggest ethanolic extract and aethyl acetate (acid) fraction at dose 1,2 g kg/bw of Mengkudu (Morinda citrifolia L.) fruits are the potential use in therapy of hyperglycemia.

Keywords: Hypoglycemic activity, Morinda citrifolia L., fruits, Glucose Tolerance Test.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, caused by lack of insulin production, insulin resistance, or both. It is generally classified into type 1 and 2 diabetes. Type 1 diabetes is insulin dependent for life (5-10%), occurs because of β pancreas cells damage. While type 2 diabetes (90-95%) occurs due to insulin resistance, insufficient production of insulin, or both [1]. This disease can also cause chronic complications include abnormalities in micro vascular and macro vascular [2]. In Indonesia, the prevalence of diabetes in adulthood, 20-
79 years, was 5.8% of the 9 million total diabetic cases and 4.8 million undiagnosed diabetic cases in 2014 [3]. Oral hypoglycemic drugs, such as biguanide, sulfonylurea and tiazololidindion, are used clinically in the treatment of type 2 diabetes, but it may cause adverse effects and become ineffective in long diabetic treatment. Complementary treatment for dibetic using medicinal plants to be the preferred solution as an alternative. Mengkudu (Morinda citrifolia L.) fruit is traditionally used for the treatment of Diabetes. The World Health Organization recommends using of traditional medicine with basic material of plants for diabetes treatment [4]. Alternative measures using herbal medicine agents may also be considered in helping to lower blood glucose levels, with minimal side effects and low cost [5]. Some studies related to the activity of antidiabetic that the extract of ethanol of mengkudu fruit showed significant can decreasing blood glucose level activity in mice induced alloxan [6] Indonesia is a country rich in biodiversity, especially with regard to medicinal raw materials. It has 30,000 flowering plants species [6], about 7000 of these species are recognized as medicinal plants [7], 950 species are discovered to have pharmacological properties, 283 species are registered, being cultivated and used by industries [8] and another species directly harvested from forest as raw material [9]. Many plants have potential as antidiabetic agent that can be proven through ethno botany information report [10]. This study will evaluate hypoglycemic activity of ethanolic extract and fraction of Mengkudu (Morinda citrifolia L.) .Hypoglycemic activity test was conducted in healthy Wistar rats, with glucose tolerance test. The ethanolic extracts with three variation doses at 0,3; 06 and 1,2 g /kg bw and the same doses 1,2/kg bw of water, aethyl acetate (base), aethyl acetate (acid), and n-hexane fraction of Mengkudu (Morinda citrifolia L) fruits given orally. The blood samples were used for determination of glucose level examination using spectrofotometric with GOD-PAP methode, in order to determine ethanolic extract and friction with the most potential as a hypoglycemic agent.

METHODS

Plant materials
The Plant materials were collected from Lembang, West Java; The plants were authenticated at Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biological Science, UNPAD.

Plant extraction
Plant materials were dried and ground, extracted by maceration method in 70% ethanol and concentrated using rotary evaporator under reduced pressure. The ethanolic extract was dissolved in aquadest, inserted into separator funnel, and then added n-hexane solvent with the same volume. The mixture is shaken for a while, then left until the two phases are separated. After separating, the n-hexane phase is accommodated in a beaker glass. The fractionation is followed by the addition of an ethyl acetate phase into a separating funnel containing a water phase in an acidic atmosphere (with the addition of HCl to pH 4). Then the acid ethyl acetate phase is accommodated in a glass beaker. Then the subsequent fraction by using an ethyl acetate solvent in an alkaline atmosphere (with the addition of NaOH to pH 8). Then performed the same separation as in the previous separation. Each fraction obtained is then evaporated and thickened by evaporating the solvent.

Animal preparation
The animal house ITB supplied male Wistar rats, weighing 200-220 g. They were housed under standard laboratory condition at room temperature of 22±3 ºC and relative humidity of 50-60%, with 12 hours light and dark cycle. Food and water were provided ad libitum according to
laboratory standard. Ethical clearance for experimental animals using was obtained from Ethical Committee of Medical Faculty UNPAD, with Ethical Approval No. 1097/UN6.C1.3.2/KEPK/PN/2017

Hypoglycemic Activity Assay
Oral glucose tolerance test was used to evaluate the hypoglycemic activity of ethanolic extracts and fraction of Mengkudu (Morinda citrifolia L) fruits given orally. Rats were divided 6 groups (3 rats per group), previously been fasted for 18 hours. Normal control group was given PGA 2%. Negative Control group was given PGA 2% and glucose 2 g/kg bw. Test group were given PGA 2%, glucose 2 g/kg bw and ethanolic extracts tested with three variation doses at 0,3 g/kg bw (DI); 06 g/kg bw (DII) and 1,2 g/kg bw (DIII) given orally and for fraction rats were divided 7 groups (3 rats per group), previously been fasted for 18 hours. Normal control group was given PGA 2%. Negative Control group was given PGA 2% and glucose 2 g/kg bw. Test group were given PGA 2%, glucose 2 g/kg bw and at the same doses 1,2/kg bw of water (T I), ethyl acetate (base) (T II), ethyl acetate (acid) (T III) and n-hexane (T IV) fraction of Mengkudu (Morinda citrifolia L) fruits given orally. Blood sampling performed at 30 minutes after plant extract was given (t = 0), then at 30 minute (t = 30), 60 minute (t = 60), 90 minute (t = 90) and 120 minute (t =120) after administration of glucose, determination of glucose level using spectrofotometric with GOD-PAP methode.

RESULTS AND DISCUSSION
The hypoglycemic activity of ethanolic extracts of Mengkudu (Morinda citrifolia L) fruits with three variation doses at 0,3; 06 and 1,2 g/kg bw orally on blood glucose levels in glucose induced rats presented by average relative blood glucose (%) as shown in Figure 1 and tabel 1.

![Figure 1: Average relative blood glucose level (%) of ethanolic extracts of Mengkudu fruits](image-url)
with three variation doses at 0.3; 0.6 and 1.2 g/kg bw orally were tested for their hypoglycemic activity using glucose tolerance test.

Table 1. Average relative blood glucose level (%) of ethanolic extracts of Mengkudu (Morinda citrifolia L.) fruits with three variation doses at 0.3; 0.6 and 1.2 g/kg bw orally were tested for their hypoglycemic activity using glucose tolerance test.

<table>
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<th>Time</th>
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<th>C(-)</th>
<th>C(+)</th>
<th>D I</th>
<th>D II</th>
<th>D III</th>
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<td>100</td>
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*Significant compared to negative control (P<0.05)

CN   : PGA 2%
C(-)  : PGA 2% + glucose 2 g/kg bw
C(+)  : PGA 2% + metformin 0.1 g/kg bw + glucose 2 g/kg bw
D I   : PGA 2% + ethanolic extract 0.3 g/kg bw + glucose 2 g/kg bw
D II  : PGA 2% + ethanolic extract 0.6 g/kg bw + glucose 2 g/kg bw
D III : PGA 2% + ethanolic extract 1.2 g/kg bw + glucose 2 g/kg bw

To obtain the best hypoglycemic activity of ethanolic extract Mengkudu (Morinda citrifolia L.) fruits from each mouse group was done with ANOVA test followed by Newman-Keuls test base on Table 1. With ANOVA test result that each treatment group each dose of ethanolic showed activity to decrease blood glucose level at 99% confidence level and based on the Newman-Keuls test that the three dose of ethanolic extract showed decrease on blood glucose level in rats induced glucose compared with negative control, and dose 1.2 g/kg bw showed the best activity to decrease blood glucose level followed by dose 0.6 g/kg bw and dose 0.3 g/kg bw. also shown in Figure 1.

Hypoglycemic activity of the same doses 1.2/kg bw of water, ethyl acetate (base), ethyl acetate (acid), and n-hexane fraction of Mengkudu (Morinda citrifolia L.) fruits given orally on blood glucose levels in glucose induced rats presented by average relative blood glucose (%) as shown in Figure 2 and Table 2.
Figure 2. Average relative blood glucose level (%) of the same doses 1,2/kg bw of water, aethyl acetat (base), aethyl acetate (acid), and n-hexane fraction of Mengkudu (Morinda citrifolia L.) fruits given orally were tested for their hypoglycemic activity using glucose tolerance test.

Table 2. Average relative blood glucose level (%) of the same doses 1,2/kg bw of water, aethyl acetat (base), aethyl acetate (acid), and n-hexane fraction of Mengkudu (Morinda citrifolia L.) fruits given orally were tested for their hypoglycemic activity using glucose tolerance test.

<table>
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<th>Waktu</th>
<th>CN</th>
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<th>C(+)</th>
<th>T I</th>
<th>T II</th>
<th>T III</th>
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</tr>
</tbody>
</table>

*Significant compared to negative control (P<0.05)

CN : PGA 2%
C(-) : PGA 2% + glucose 2 g/kg bw
C(+) : PGA 2% + klorpropamid 22.5 mg/kg bw + glucose 2 g/kg bw
T I  : PGA 2% + water fraction 1.2 g/kg BB + glucose 2 g/kg bw
T II : PGA 2% + aethyl acetate (base) fraction 1.2 g/kg bw + glucose 2 g/kg bw
T III : PGA 2% + aethyl acetate (base) fraction 1.2 g/kg bw + glucose 2 g/kg bw
T IV : PGA 2% + n-hexane fraction 1.2 g/kg bw + glucose 2 g/kg bw

Also to obtain same doses 1,2/kg bw the best hypoglycemic activity of water, aethyl acetat (base), aethyl acetate (acid), and n-hexane fraction of Mengkudu (Morinda citrifolia L.) fruits given orally from each rats group was done with ANOVA test followed by Newman-Keuls test base on Table 2. The results of ANOVA test showed that each of the treatments in this experiment, normal control group, positive control, negative control and test group of each fraction gave significant difference to the decrease of rat blood glucose level with 95% confidence level. The results of Newman-Keuls test showed that test that water, aethyl acetat
(base), ethyl acetate (acid), and n-hexane fraction showed decrease on blood glucose level in rats induced glucose compared with negative control with a significant level of 0.05 and fraction having the best activity of decreasing the blood glucose level are the ethyl acetate (acid) fraction followed by the water fraction, the ethyl acetate (base) fraction and n-hexane fraction also shown in Figure 2. Morinda citrifolia fruit juice gave better result to the decrease of rat blood glucose level in rat induced alloxan [11] The hypoglycemic effect of M.citrifolia roots in STZ-induced diabetic mice is due to the presence of anthraquinones. In particular, the anthraquinones that have no substituents on one aromatic ring seem to play an important role in the hypoglycemic effect of M. citrifolia. [12].

CONCLUSION
The ethanolic extract of Mengkudu (Morinda citrifolia L.) fruits has the best activity in reduction of blood glucose level in rats at doses 1,2 g/kg bw, followed by doses 0,6 g/kg bw and doses 0,3 g/kg bw and also showed that the ethyl acetate fraction (acid) gave the best activity in reduction of blood glucose level in rats (54,29%), followed by water fraction (47,42%), ethyl acetate (base) fraction (44,51%) and n-hexane fraction (34,18%). The present study suggest ethanolic extract and ethyl acetate (acid) fraction at dose 1,2 g kg/bw of Mengkudu (Morinda citrifolia L.) fruits are the potential use in therapy of hyperglycemia.

REFERENCES